Analysis of species lineages of some Australian thiarids (Thiaridae, Prosobranchia, Gastropoda) using the evolutionary species concept

James A. Stoddart[1]
Department of Zoology
University of Western Australia
Nedlands, Western Australia 6009

[1]. Present Address: Australian Institute of Marine Science
PMB No. 3, TOWNSVILLE MC, QLD 4810

ABSTRACT

The evolutionary species definition is applied to 16 populations of Australian thiarids through the numerical analysis of an electrophoretic data set. Thirteen populations were assigned to a single lineage on the basis of their common evolutionary past and their evolutionary disjunction from the remaining three populations. Members of this lineage were referred to the species *Thiara balonnensis* (Conrad, 1850). The relationships of the other three populations, which were tentatively assigned to *Thiara denisoniensis* (Brot, 1877), were uncertain apart from being outside this lineage.

INTRODUCTION

A fundamental barrier to the natural (sensu Gilmour, 1961) classification of the Thiaridae is presented by the parthenogenetic mode of reproduction found in many species (Jacob, 1957a & b). Asexually reproducing organisms may produce patterns of variation which are confusing to taxonomists and in general, their delimitation into species falls outside the limits of the biological species concept of Mayr (1963). As asexual reproduction does not permit recombination or reassortment of genes, numbers of apparently independent characters will show a close association within a clone. This will frequently lead to the production of distinctive forms which may be quite dissimilar to other clones, resulting in the recognition of a clone as a named species. A more appropriate classification of distinctive clones may become apparent if many clones are compared simultaneously. However, the criteria to be used in this classification can obviously not be based on reproductive isolation. We require a species concept which is relevant to asexual reproduction.

Wiley's (1978) modification of Simpson's (1961) evolutionary species concept stresses the relevance of the process of speciation to species definitions and provides the most appropriate framework

for the taxonomy of asexual organisms. Here, a species is defined as "a single lineage of ancestral-descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate" (Wiley, 1978, p18). Although Simpson's (1961) earlier definition had been criticised for its lack of operationalism (Fritts, 1962; Sokal and Crovello, 1970), Wiley's implied criterion of evolutionary disjunctions between species (Wiley, 1981) seems well suited to the application of numerical methods used in the estimation of evolutionary lineages.

Ideally, the construction of lineages should employ a large number of phenotypic characters, and in particular, those characters which are able to be related directly to the genotype. While it is rarely possible to weigh changes in phenotypic characters in a precise quantitative relationship with their corresponding genotypic changes, it would be wrong to ignore the parameters of the genotype-phenotype relationship entirely. This would invite disproportionate influence of a few characters on the resultant phylogeny estimate. A working compromise is to select a set of characters which are likely to have equivalent amounts of genotypic evolution implied by their changes in state. In this case, similarities in the ontogeny, non-genetic components, and coding of characters should be used in the choice of characters.

Avise (1974) lists some advantages of electrophoretic characters over morphological, or anatomical, characters in the above respect, the most important being their equivalent amounts of genotypic information. Electrophoretic characters were used here, as in terms of genotypic change it is easier to relate a change in allozyme state of one enzyme to that of another enzyme than to compare, say a change in shell length with one of shell sculpture. This does not imply that electrophoretic data will be inherently superior to morphological data in systematic inference. Rather it is the ease with which these data are interpreted which recommends them (see Mickevich and Johnson, 1976, p 268, for a discussion of "data" versus "ease").

Australian thiarids have been referred to a large number of named forms (Iredale, 1943), frequently on the basis of a few morphological characters, and frequently to forms with very limited distribution. The single study which has attempted to compare a number of these "species" (Blackwell, 1969) was unable to reach a definite conclusion as to their validity and preferred to lump them into a single taxon. This situation is symptomatic of thiarids worldwide, and a study by Reich (1937) synonymised a large proportion of the 114 named species treated by him. This apparent overnaming suggests that the application of the evolutionary species concept to thiarids may prove most effective.

This study analyses relationships within a set of 16 Australian thiarid populations. Electrophoretic characters are used as the basis for an assessment of overall similarity between the clones of these populations, i.e. a phenetic classification, and to reconstruct the evolution of these forms, i.e. a phylogenetic classification. This allows both the assessment of their present relationships and the examination of the likely pathways which lead to these relationships. In this way, it should be possible to detect any disjunctions in their evolution.

MATERIALS AND METHODS

Sample material: Fourteen localities were chosen for inclusion in this study such that certain subsets of the 14 represented a) an extensive geographic range within Australia, b) a contiguous, or nearly so, distribution, c) morphologically distinct populations, and d) a wide range of habitats. Sample localities (Fig. 1) followed in parentheses by 3-letter codes and the catalogue numbers of voucher specimens lodged at the Western Australian Museum were: Lake Leschenaultia (LES : 471-80), Swan River (MSW : 680-79), Ellendale Pool (ELT : 461-80 & ELM : 465-80), Fortescue Falls (TFT : 469-80 & DFT : 466-80), Fitzroy River (FIZ : 459-80) and Ord River (KUN : 457-80) all of Western Australia; Victoria River (VIC : 458-80) and Finke River (FIN : 470-80) of the Northern Territory; Brisbane River (BAL : 463-80) and Tinaroo Dam (STA : 464-80) of Queensland, and Lake Liddell (LID : 460-80) in New South Wales. Exact locality data are given in Stoddart (1980).

Only at two sites were there more than a single morphotype apparent. At these sites, several shell characters showed clearly bimodal distributions (Stoddart, 1980) and as morphotypes from each site were initially referred to different named forms, snails were subdivided into populations on this basis (viz. ELT/ELM and TFT/DFT).

Using existing specific classifications based on shell characters and the generic scheme of Pace (1973) and Stoddart (1980) the 16 populations were referrable to six taxa on the basis of the comparisons with descriptions and figures of types:

Thiara (Thiara) balonnensis (Conrad, 1850) — BAL, LID
Type locality — Balonne River, Australia
T. (T.) tetrica (Conrad, 1850) — FIN, CPM
Type locality — Murray River, S.E. Australia
T. (T.) australis (I. & H. Lea, 1850) — KUN, VIC, MNP, TFT, FIZ
Type locality — Victoria River, Northern Territory
T. (T.) incerta (Brot, 1862) — MSW, LES, ELT, MUR
Type locality — Avon River, Western Australia
T. (Melanoides) denisoniensis tacita (Iredale, 1943) — STA
Type locality — Cardwell, Queensland
T. (M.) d. ultra (Iredale, 1943) — ELM, DFT
Type locality — Clarence River, New South Wales.

Type localities given for many of the species of Australian thiarids are often vague and sometimes misleading. For example, the species *denisoniensis* (Brot, 1877) has Port Denison, Qld as its given locality, even though this is a marine embayment and more likely to be the port where specimens were loaded onto a ship, rather than where they were found. Others are from rivers which stretch for hundreds of kilometres. Thus it is extremely difficult to be certain whether snails were collected from type localities. Of the present populations, VIC and MSW were collected from the same river systems as the types of their assigned species. With the exception of ELM and DFT, other populations occurred in the same broad geographic region as the types of their assigned species.

Electrophoresis: Horizontal starch gel (12% Electrostarch, Otto Hiller & Co, Lot 307) electrophoresis was performed using refrozen foot tissue homogenised the previous day. Snails were frozen and stored at -20°C for between 1 day and 6 months prior to being homogenised. Controls indicated no apparent effects of freezing or storage on banding patterns. Gels were stained for the following enzymes: esterase (EST), leucyl-glycylglycine peptidase (LGG), leucyl-proline peptidase (LP), leucyl-tyrosine peptidase (LTY), glutamate oxaloacetate transaminase (GOT), leucine aminopeptidase (LAP), nucleoside phosphorylase (NP), phosphoglucomutase (PGM), 6-phosphosgluconate dehydrogenase (6PGD), malate dehydrogenase (MDH), mannose phosphate isomerase (MPI) and superoxidase dismutase (SOD). All 4 LGG bands correspond to bands with identical mobility in LTY and were presumed to result from non-specific allozymes, that is, enzymes which metabolise a number of different substrates. Each allozyme was scored as a single character.

Confident interpretation of banding patterns into locus-allele models relies on determining patterns of inheritance of bands from breeding experiments. As this was not possible for an obligate parthenoform, each band was considered a discrete character and scored on a presence/absence basis. Bands were labelled alphabetically in order of decreasing anodal mobility.

Numerical methods:

1) *Phenetic*: Presence/absence characters were used to compute a matching coefficient of similarity, Jaccard's Sj (Sneath & Sokal, 1973). This measure of overall similarity was then used in the UPGMA algorithm (Sokal & Michener, 1958) to sequentially cluster the populations and produce a phenogram showing their relationships.

2) *Phylogenetic:* An unrooted Wagner tree (Farris, 1970), or network, was computed using the Wagner78 program of Farris, run on the Western Australian Regional Computing Centre's Cyber 76. Wagner methods are a form of cladistic analysis which attempt to specify the evolutionary pathway between a series of taxa by searching for the path requiring the least amount of evolution. Essentially this means that path which minimises the number of times any character is derived, i.e. the parsimony criterion (Felsenstein, 1983). The distance between each taxon on the tree is called the patristic distance and was defined as the total number of divergent, convergent and parallel evolutionary steps between each, with minimal estimates of the latter two produced under the parsimony criterion.

RESULTS

After correcting for non-specific enzymes, 46 scoreable characters remained (Table 1). No variation in banding pattern was seen within any population, suggesting that each contained only a single clone of genetically identical snails. With the exception of the MSW-LES pair, no two populations shared the same clone. The LES population occurs in a man-made empoundment and is almost certainly derived from snails originating from the same river as MSW. Differences between clones were extensive with Sj's from 0.98 to 0.55 (Table 2).

The topologies of the phenogram (Fig.2) and Wagner tree (Fig.3) are largely concordant. Both show that for the majority of populations, electrophoretic estimates of genetic distance are similar to amounts of geographic separation. When DFT, ELM and STA are excluded, both Sj and patristic distance (PD) are significantly correlated with differences in latitude between populations (Table 3). Both correlations become nonsignificant on inclusion of DFT, ELM and STA, and predictions of genetic distances between these and the other thirteen populations, using the relationship derived above, typically underestimate the actual values. Regression statistics are shown in Table 3.

DISCUSSION

A necessary corollary to the search for an evolutionary disjunction between lineages is that there must be an ordered element to evolution within a lineage. Such an element has been previously reported for this group in the context of the evolution of an asexual organism (Stoddart, 1983). It is seen here in the relationship between a geographic parameter (latitude) and genetic distance measures (Sj and PD). Presumably this reflects the influence of the dispersal process on the time elapsed since clones shared a common ancestor (Stoddart, 1983). Thus for the 13 populations where this relationship holds, a common evolutionary tendency is apparent in that there has been no substantial alteration of characteristics affecting evolutionary response. Included here are such features as generation time, ploidy and niche. The breakdown of this relationship when STA, DFT and ELM are included and the extent of genetic differences between these and the other 13 suggest the presence of an evolutionary disjunction between the main lineage and these three clones.

At each site, new clones will be produced by mutation with subsequent competition between these and extant clones resulting in a reduction in clonal diversity (Jaenike *et al.*, 1980). Additionally, Livshits and Fishelson (1983) show that some populations of *Thiara tuberculata*, a species previously thought to be entirely parthenogenetic, reproduce sexually. Patchy occurrences of sexual reproduction, whether temporally or spatially patchy, will also generate new clones in apparently asexual species of thiarids. The number of clones present at each site will depend then on the balance between the rate of viable mutations, or the frequency of sex, and the intensity of interclonal competition. The uniclonal nature of most sites suggests the rate of clonal production to be lower than the rate of clonal extinction. Thus for two clones to persist in time they must be geographically separated or occupy largely non-overlapping niches. Clones of the main lineage provide an example of the former category and the ELM-ELT and DFT-TFT clone pairs an example of the latter. The sympatric occurrence of these latter clones strengthens the previous conclusion that members of each pair belong to separate lineages (species), thus having distinct niches and divergent evolutionary tendencies.

It is not the sympatric occurrence of ELM and DFT with members of the main lineage that alone dictates their separation from this lineage. Rather, it is their disjunction from the genetic-geographic association characterising the main lineage. It would be possible for two clones to exist sympatrically and be quite distinct, yet to be referable to a single lineage when compared with adjacent members of that lineage. Similarly, while no members of the main lineage occur sympatrically with STA, this population can still be separated from this lineage on the basis of its much greater genetic distance from members of the lineage than that predicted from its geographic position.

Thirteen populations, LES, MSW, ELT, MUR, CPM, MNP, TFT, FIZ, KUN, VIC, FIN, BAL and LID, then may be assigned to a single species. By virtue of priority of publication the name for this species should be *Thiara (T). balonnensis* (Conrad, 1850), this being the most senior of the four

names represented by this group. Although neither BAL nor LID, the populations originally referred to *T. balonnensis* on morphological grounds, are from this species' type locality, it seems unlikely that a population which occurs not far from these two and is so similar morphologically would form part of a separate lineage. Asexual groups are conservative with respect to speciation (Stanley, 1979) and further study may show *T. balonnenis* to be conspecific with a more cosmopolitan species such as *T. scabra* or *T. tuberculata*. However, the appropriate answer to this question requires the comparison of series of populations from all three species.

Of the taxa not included in the *T. balonnensis* lineage, both phenogram and Wagner tree suggest a taxonomic separation of STA from DFT and ELM in excess of the subspecies level produced by Iredale (1943). This is apparent in the substantial genetic distance between these two groups, vis-a-vis distances between members of the main lineage. However, further populations of these species are needed to clarify their status by determining if they are part of a single lineage or not. Although it is certain they are not conspecific with *T. balonnensis*, there is insufficient information to comment on their placement in a separate subgenus. In the absence of substantive evidence, these forms are left in the taxa to which they have been assigned already.

Although the application of the evolutionary species definition is cumbersome because series of populations are needed to discern lineages, it is preferable to taxonomic criteria based on reproductive isolation. In the present context it has demonstrated its utility by showing that order exists within a set of phenotypically disparate populations in a troublesome group. Its use for the parthenogenetic thiarids may remedy the apparently gross overnaming of species which has stemmed from an inability to deal with discrete clonal variation.

ACKNOWLEDGEMENTS

This study formed part of an M.Sc. within the University of Western Australia. I thank Dr M.S. Johnson for guidance during the project and for comments on the manuscript. Alan Lymbery also helped with suggestions on the paper. I am indebted to the following collectors of snails: J.B. Burch, J.C. Pearson, G. Strickland and J. Walker. Work was supported by research grants from the University of Western Australia and the Western Australian Department of Public Health.

LITERATURE CITED

Avise, J.C., 1974. Systematic value of electrophoretic data. Syst. Zool., 23 : 465-481.

Blackwell, J., 1969. *Plotiopsis australis* in the Swan River Estuary Honours Thesis, University of Western Australia, Perth.

Farris, J.S., 1970. Methods for computing Wagner trees. Syst. Zool., 19 : 83-92.

Felsenstein, J., 1983. Parsimony in systematics : Biological and statistical issues. Ann. Rev. Ecol. Syst., 14 : 313-333.

Fritts, T.H., 1969. The systematics of the parthenogenetic lizards of the *Cnemidophorus cozumela* complex. Copeia, 1969 : 519-535.

Gilmour, J.S.L., 1961. Taxonomy. In: McLeod, A.S. & Cobley, L.S., (Eds.) Contemporary Botanical Thought, Quadrangle Books, Chicago, pp 27-45.

Iredale, T., 1943. A basic list of the freshwater Mollusca of Australia. Aust. Zool., 10 : 188-230.

Jacob, J., 1957a. Cytological studies of Melaniidae (Mollusca) with special reference to parthenogenesis and polyploidy. I. Oogenesis of the parthenogenetic species of *Melanoides*. Trans. Roy. Soc. Edin., 63 : 341-352.

Jacob, J., 1957b. Cytological studies of Melaniidae (Mollusca) with special reference to parthogenesis and polyploidy. II. A study of meiosis in the rare males of the polyploid race of *Melanoides tuberculata*. Trans. Roy. Soc. Edin. 63 : 433-444.

Jaenike, J., Parker, E.D. & Selander, R.K. 1980. Clonal niche structure in the parthenogenetic earthworm *Octolasion tyrtaeum*. Amer. Natur. 116 : 196-205.

Livshits, G. & Fishelson, L., 1983. Biology and reproduction of the freshwater snail *Melanoides tuberculata* (Gastropoda : Prosobranchia) in Israel. Israel J. Zool., 32 : 21-35.

Mayr, E., 1963. Animal Species and Evolution. Belknap Press, Cambridge.

Mickevich, M.F. & Johnson, M.S., 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. Syst. Zool., 25 : 260-270.

Pace, G.L., 1973. Freshwater snails of Taiwan (Formosa). Mal. Res. Suppl., 1 : 1-118.

Riech, E., 1937. Systematische anatomische, okologische und tiergeographische Untersuchungen uber der Susswasser Mollusken Papuasiens und Melanesiens. Arch. Natur., 6 : 37-153.

Simpson, G.G., 1961. Principles of Animal Taxonomy. Columbia University Press, New York.

Sneath, P.H.A. & Sokal, R.R., 1973. Numerical Taxonomy. W.H. Freeman & Co., San Francisco.

Sokal, R.R. & Crovello, T.J., 1970. The biological species concept : a critical evaluation. Amer. Natur., 104 : 127-153.

Sokal, R.R. & Michener, C.D., 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull., 38 : 1409-1438.

Stanley, S.M., 1979. Macroevolution. W.H. Freeman & Co., San Francisco.

Stoddart, J.A., 1980. Taxonomy and evolutionary potential of a parthenogenetic freshwater prosobranch. M.Sc. Thesis, University of Western Australia, Perth.

Stoddart, J.A., 1983. The accumulation of genetic variation in a parthenogenetic snail. Evolution, 37 : 546-554.

Wiley, E.O., 1978. The evolutionary species concept reconsidered. Syst. Zool., 27 : 17-26.

Wiley, E.O., 1981. Phylogenetics : The theory and practice of phylogenetic systematics. John Wiley and Sons, New York.

TABLE 1. Clonal structure of the 16 study populations. Each letter denotes the presence of that band in a clone. Bands appearing for both LTY and LGG were LTY, a,d,f and h. N is the sample size in each case.

			Enzyme									
	Clone	**N**	**LTY**	**LP**	**LAP**	**EST**	**MPI**	**PGM**	**6PGD**	**MDH**	**NP**	**GOT**	**SOD**
1.	MSW/LES	40/15	adefh	bc	acd	bdegh	bcd	adefg	abc	cd	a	a	ab
2.	ELT	10	adefh	bc	acd	abdegh	abcd	abdefh	abc	bd	a	a	ab
3.	MUR	20	adefh	bc	acd	bdegh	acd	abdefg	b	bd	a	a	ab
4.	LID	9	adefh	bc	cd	bdegh	ac	befg	ab	de	a	a	ab
5.	CPM	10	adefh	bc	acd	abdegh	acd	bdefg	bc	abcde	a	a	ab
6.	MNP	10	adeh	bc	ac	abdegh	acd	bdef	bc	bc	a	a	ab
7.	TFT	6	adeh	bc	ac	abdegh	acd	bdf	ac	abcde	a	a	ab
8.	BAL	10	adeh	bc	ac	bdgh	ac	befg	c	abcde	a	a	ab
9.	FIN	10	aeh	c	ac	adegh	ac	bcefg	b	abcde	a	a	ab
10.	VIC	8	adeh	a	c	abdgh	ac	bef	bd	bd	a	a	ab
11.	KUN	26	abeh	a	c	adgh	ac	bef	ac	bd	a	a	ab
12.	FIZ	6	abeh	a	c	adgh	ac	bcef	ab	bd	a	a	ab
13.	ELM	8	bdfgh	c	bc	adgh	ab	bg	ab	bde	a	a	ab
14.	DFT	12	bdfgh	bc	bc	dgh	ab	bd	abc	bde	a	a	ab
15.	STA	4	acdgh	a	bc	bcdfh	a	abdf	d	abde	a	a	ab

TABLE 2. Matrix of Jaccard's S_j as a measure of genetic similarity, based on one band equals one character.

ELT	.92													
MUR	.89	.98												
LID	.85	.88	.90											
CPM	.86	.91	.91	.88										
MNP	.83	.89	.88	.84	.92									
TFT	.79	.85	.80	.79	.91	.81								
BAL	.76	.79	.81	.84	.89	.86	.88							
FIN	.69	.76	.77	.80	.86	.82	.81	.85						
VIC	.67	.79	.76	.75	.79	.81	.76	.80	.76					
KUN	.64	.75	.69	.71	.76	.74	.76	.76	.72	.91				
FIZ	.60	.72	.69	.71	.68	.70	.69	.68	.80	.87	.91			
ELM	.69	.76	.67	.62	.69	.64	.67	.63	.63	.64	.68	.64		
DFT	.65	.69	.66	.64	.65	.59	.62	.62	.58	.58	.63	.63	.69	
STA	.55	.60	.63	.62	.63	.57	.60	.67	.59	.68	.64	.60	.65	.69
	MSW LES	ELT	MUR	LID	CPM	MNP	TFT	BAL	FIN	VIC	KUN	FIZ	ELM	DFT

TABLE 3. Regression statistics for the relationship of genetic and geographic distance, of the form $Y = bX + a$, where X is the difference in latitude between populations (standardised by division by the grand mean).

* - $P < 0.5$; ** - $P < .01$ for 11 d.f.

Clones in the Regression	Y	a	b	r	R^2
All - (ELM, DFT, STA)	S_j	.87	-.24	-.57*	.32
All	S_j	.78	-.14	-.24	.06
All - (ELM, DFT, STA)	PD	8.08	28.5	.67**	.45
All	PD	16.3	19.0	.32	.09

Figure 1: Map of Australia showing the sample sites.

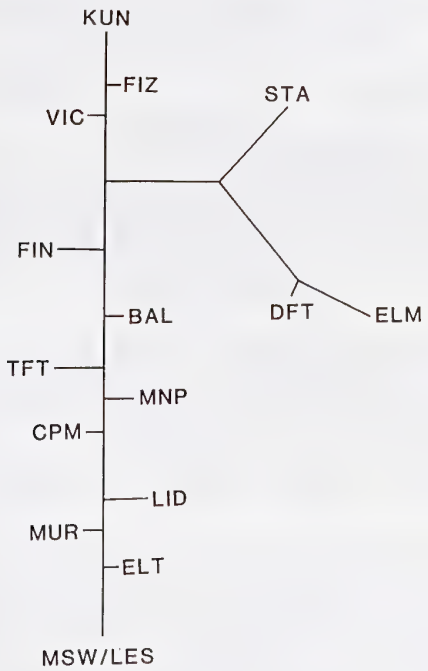

Figure 2: Phenogram resulting from UPGMA clustering based on S_j values.

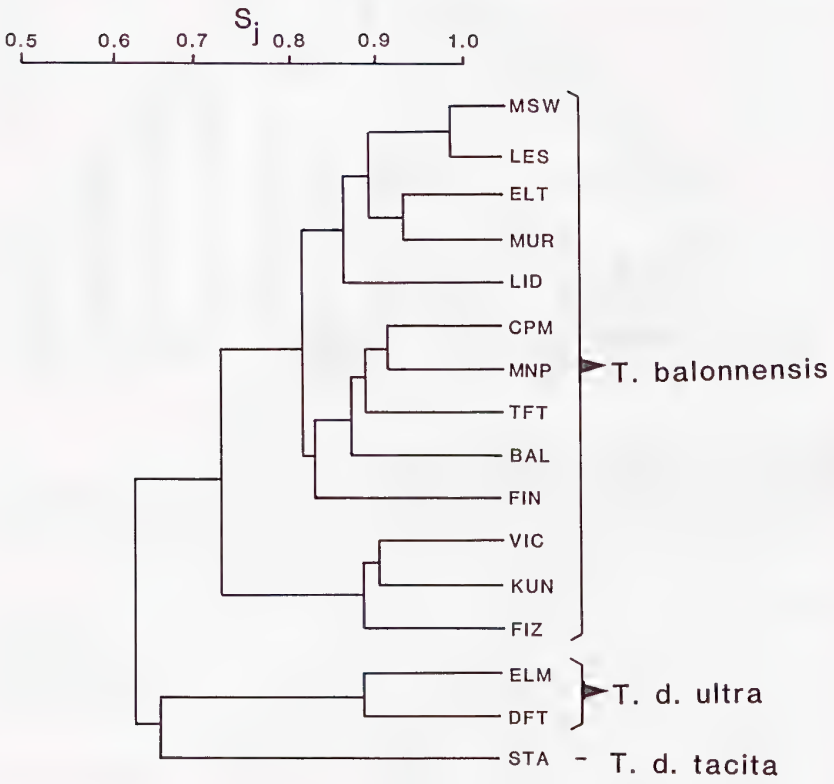

Figure 3: Unrooted Wagner tree. Internode distances are proportional to patristic distances.